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L2: Entry 1 of 1 File: USPT Mar 12, 2002

DOCUMENT-IDENTIFIER: US 6355425 B1
TITLE: Mutations associated with iron disorders

US Patent No. (1):
6355425

Detailed Description Text (14):
A biological sample containing RNA or DNA is obtained from an individual and the nucleic acid extracted. Optionally, the nucleic acid is amplified according to standard procedures such as PCR. A nucleic acid polymorphism, e.g, a single base pair polymorphism, is detected using methods well known in the art of molecular biology. For example, a mutation is detected using a standard sequencing assay, nucleic acid hybridization, e.g, using standard Southern, Northern, or dot blot hybridization assay systems and an HFE-specific oligonucleotide probe, restriction enzyme fragment polymorphism analysis, oligonucleotide ligation assay (OLA; Nikerson etal., 1990, Nucl. Acids Res. 87:8923-8927), primer extension analysis (Nikiforov etal., 1994, Nucl. Acids Res. 22:4167-4175), single strand conformation polymorphism (SSCP) analysis, allele-specific PCR (Rust etal., 1993, Nucl. Acids Res. 6:3623-3629), denaturing gradient gel electrophoresis (DGGE), fluorescent probe melting curve analysis (Bernard et al., 1998, Am. J. Pathol. 153:1055-61), RNA mismatch cleavage assay, capillary hybridization, or TaqMan.TM. assay (PE Applied Biosystems, Foster City, Calif.). Nucleic acid hybridization assays are also carried out using a bioelectronic microchip technology known in the art, e.g., that described in Sosnowski etal., 1997, Proc. Natl. Acad. Sci. U.S.A. 94:1119-1123; Cheng etal. 1998, Nature Biotechnology 16:541-546; or Edman etal., 1997, Nucl. Acids Res. 25:4907-4914.

Detailed Description Text (29):
To detect mutations in exon 2 of the HFE gene, the genomic DNA of probands and normal control subjects were amplified and subjected to a dot blot hybridization assay. 1.0 .mu.l of each resulting PCR product was then applied to a Magna Graph nylon membrane (MSI, Westboro, Mass.). The membranes were treated with 0.5 N NaOH/1.5 M NaCl to denature the DNA, neutralized with 0.5 M Tris-HCl (pH 8.0)/1.5 M NaCl, and rinsed with 2.times.SSC (1.times.SSC=0.15 M NaCl/0.015 M sodium citrate, pH 7.0). The DNAs were fixed on the membrane by UV irradiation using a Stratalinker 1800 (Stratagene, Inc., La Jolla, Calif.). The ECL 3'-oligolabelling and detection system (Amersham, Arlington Heights, Ill.) was used for synthesis of labeled oligonucleotide probes, hybridization, and signal detection. The oligonucleotide sequences used to detect each point mutation were (substituted bases are shown as upper case letters):

Detailed Description Text (30):
For signal detection, each oligonucleotide was labeled with fluorescein-11-dUTP using terminal deoxynucleotidyl transferase according to the manufacturer's instructions (Amersham Ltd., Arlington Heights, Ill.). The membranes were prehybridized in 5.times.SSC, 0. 1% Hybridization buffer component, 0.02% SDS, 5% LiquidBlock at 42.degree. C. for approximately 2 hours. Labelled oligonucleotide probes were added to individual bags containing the membranes and prehybridization buffer and incubated at 42.degree. C. overnight. The blots were washed twice with 5.times.SSC, 0.1% SDS for 5 minutes at room temperature. Stringency washes for hybridization with oligonucleotides having the sequence of SEQ ID NO:30 or 28 were performed twice in 0.2.times.SSC/0.1% SDS for 15 minutes at 42.degree. C. Membranes probed with an oligonucleotide having the sequence of SEQ ID NO:29 was washed twice under less stringent conditions

(0.5.times.SSC/0.1% SDS, 15 minutes at 42.degree. C.). Detection of a fluorescent signal was performed according to standard methods.

Other Reference Publication (5):
Bernard et al., Homogeneous Multiplex Genotyping of Hemochromatosis Mutations with Fluorescent Hybridization Probes, Am. J. Pathology, vol. 153, No. 4, Oct., 1998. pp. 1055-1061.

Other Reference Publication (9):
Edman et al., Electic field directed nucleic acid hybridization on microchips, Nucleic Acids Research, 1997, vol. 25, No. 24, pp. 4907-4914.

Other Reference Publication (10):
Cheng et al., Prepartion and hybridization analysis of DNA/RNA from E. coli on microfabricated bioelectric chips, Nature Biotechnology, vol. 16, pp. 541-546, Jun. 1998.

Other Reference Publication (29):
Edman et al., "Electric field directed nucleic acid hybidiaztion on microchips", Nucleic Acids Research, 1997, vol. 25, No. 24, 1997.

Other Reference Publication (30):
Cheng et al., "Preparation and hybridization analysis on DNA/RNA from E. coli microfabricated bioelectronic chips", Nature Biotechnology, vol. 16, No. 6, Jun. 1998, pp. 541-546.

Other Reference Publication (31):
Bernard et al., "Homogeneious Multiplex Genotyping of Hemochromatosis Mutations with Fluorescent Hybridization Probes", American Journal of Pathology, vol. 153, No. 4, 1998.

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L1: Entry 3 of 4 File: USPT Mar 12, 2002

US-PAT-NO: 6355425
DOCUMENT-IDENTIFIER: US 6355425 B1

TITLE: Mutations associated with iron disorders

DATE-ISSUED: March 12, 2002

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6; 536/22.1

CLAIMS:

What is claimed is:

1. A method of diagnosing an iron disorder or a genetic susceptibility to developing said disorder in a mammal, comprising determining the presence of a mutation in exon 2 of an HFE nucleic acid in a biological sample from said mammal, wherein said mutation is not a C.fwdarw.G substitution at nucleotide 187 of SEQ ID NO:1 and wherein the presence of said mutation is indicative of said disorder or a genetic susceptibility to developing said disorder.

2. The method of claim 1, wherein said disorder is hemochromatosis.

3. The method of claim 1, wherein said nucleic acid is a DNA molecule.

4. The method of claim 1, wherein said nucleic acid is a RNA molecule.

5. The method of claim 1, wherein said mutation is a missense mutation at nucleotide 314 of SEQ ID NO:1.

6. The method of claim 5, wherein said mutation is 314C.

7. The method of claim 6, wherein said mutation results in expression of mutant HFE gene product I105T.

8. The method of claim 1, wherein said mutation is at nucleotide 277 of SEQ ID NO:1.

9. The method of claim 8, wherein said mutation is 277C.

10. The method of claim 9, wherein said mutation results in expression of mutant HFE gene product G93R.

11. The method of claim 1, wherein said mutation is at nucleotide 193 of SEQ ID NO:1.

12. The method of claim 11, wherein said mutation is 193T.

L1: Entry 2 of 4 File: USPT Jan 21, 2003

US-PAT-NO: 6509442
DOCUMENT-IDENTIFIER: US 6509442 B1

TITLE: Mutations associated with iron disorders

DATE-ISSUED: January 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothenberg; Barry E.	Del Mar	CA	92014	
Sawada-Hirai; Ritsuko	San Diego	CA	92130	
Barton; James C.	Birmingham	AL	35209	

US-CL-CURRENT: 530/300; 435/6, 530/350

CLAIMS:

What is claimed is:

1. A substantially pure HFE polypeptide comprising amino acid substitution I105T.

2. A substantially pure HFE polypeptide comprising amino acid substitution G93R.

3. A substantially pure HFE polypeptide comprising amino acid substitution S65C.

13. The method of claim 12, wherein said mutation results in expression of mutant HFE gene product S65C.

14. The method of claim 1, wherein said biological sample is selected from the group consisting of whole blood, cord blood, serum, saliva, plasma, effusions, ascites, urine, stool, buccal tissue, liver tissue, kidney tissue, cerebrospinal fluid, skin, hair and tears.

15. The method of claim 14, wherein said biological sample is whole blood.

16. The method of claim 14, wherein said biological sample is saliva.

17. The method of claim 14, wherein said biological sample is hair.

18. The method of claim 1, wherein said mammal is a human.

19. The method of claim 1, further comprising amplifying said nucleic acid using a first oligonucleotide primer which is 5' to exon 2 and a second oligonucleotide primer is 3' to exon 2.

20. The method of claim 1, further comprising amplifying said nucleic acid using a first oligonucleotide primer which is 5' to nucleotide 314 of SEQ ID NO:1 and a second oligonucleotide primer which is 3' to nucleotide 314 of SEQ ID NO:1.

21. The method of claim 1, further comprising amplifying said nucleic acid using a first oligonucleotide primer which is 5' to nucleotide 277 of SEQ ID NO:1 and a second oligonucleotide primer which is 3' to nucleotide 277 of SEQ ID NO:1.

22. The method of claim 1, further comprising amplifying said nucleic acid using a first oligonucleotide primer which is 5' to nucleotide 193 of SEQ ID NO:1 and a second oligonucleotide primer which is 3' to nucleotide 193 of SEQ ID NO:1.

23. The method of claim 20, 21, or 22, wherein said first oligonucleotide primer has a nucleotide sequence of SEQ ID NO:3 and said second oligonucleotide primer has a nucleotide sequence of SEQ ID NO:4.

24. The method of claim 20, 21, or 22, wherein said first oligonucleotide primer has a nucleotide sequence of SEQ ID NO:15 and said second oligonucleotide primer has a nucleotide sequence of SEQ ID NO:16.

25. A method of diagnosing an iron disorder or a genetic susceptibility to developing said disorder in a mammal, comprising determining the presence or absence of a mutation in an intron of HFE genomic DNA in a biological sample from said mammal, wherein the presence of said mutation is indicative of said disorder or a genetic susceptibility to developing said disorder.

26. The method of claim 25, wherein said mutation is in intron 4.

27. The method of claim 26, wherein said mutation is at nucleotide 6884 of SEQ ID NO:27.

28. The method of claim 27, wherein said mutation is 6884C.

29. The method of claim 25, wherein said mutation is in intron 5.

30. The method of claim 29, wherein said mutation is at nucleotide 7055 of SEQ ID NO:27.

31. The method of claim 30, wherein said mutation is 7055G.

32. The method of claim 25, further comprising amplifying said nucleic acid using a first oligonucleotide primer which is 5' to intron 4 and a second oligonucleotide primer which is 3' to intron 4.

33. The method of claim 25, further comprising amplifying said nucleic acid using a first oligonucleotide primer which is 5' to intron 5 and a second oligonucleotide primer which is 3' to intron 5.

34. A method of diagnosing an iron disorder or a genetic susceptibility to developing said disorder in a mammal, comprising determining the presence of a mutation in a HFE gene product in a biological sample from said mammal, wherein said mutation results in a decrease in an intramolecular salt bridge formation in said HFE gene product but is not amino acid substitution H63D, and wherein the presence of said mutation is indicative of said disorder or a genetic susceptibility to developing said disorder.

35. The method of claim 34, wherein said disorder is hemochromatosis.

36. The method of claim 34, wherein said mutation is between amino acids 23-113, inclusive, of SEQ ID NO:2.

37. The method of claim 34, wherein said mutation is between amino acids 58-68, inclusive, of SEQ ID NO:2.

38. The method of claim 34, wherein said mutation is between amino acids 60-65, inclusive, of SEQ ID NO:2.

39. The method of claim 34, wherein said mutation is amino acid substitution S65C.

40. The method of claim 34, wherein said mutation is between amino acids 90-100, inclusive, of SEQ ID NO:2.

41. The method of claim 34, wherein said mutation is between amino acids 92-97, inclusive, of SEQ ID NO:2.

42. The method of claim 34, wherein said mutation is amino acid substitution G93R.

43. The method of claim 34, wherein said mutation is at amino acid 95 of SEQ ID NO:2.

44. The method of claim 34, wherein said mutation is detected by immunoassay.

45. A method of diagnosing an iron disorder or a genetic susceptibility to developing said disorder in a mammal, comprising determining the presence of a mutation in a HFE gene product in a biological sample from said mammal, said mutation being located in the .alpha.1 helix of said HFE gene product, wherein the presence of said mutation is indicative of said disorder or a genetic susceptibility to developing said disorder.

46. The method of claim 45, wherein said mutation is between amino acids 80-108, inclusive, of SEQ ID NO:2.

47. The method of claim 45, wherein said mutation is I105T.

48. The method of claim 45, wherein said mutation is G93R.

49. An isolated nucleic acid molecule encoding an HFE polypeptide comprising amino acid substitution I105T or the complement thereof.

50. An isolated nucleic acid molecule encoding an HFE polypeptide comprising amino acid substitution G93R or the complement thereof.

51. An isolated nucleic acid molecule encoding an HFE polypeptide comprising amino acid substitution S65C or the complement thereof.

52. A kit for detecting a nucleotide polymorphism associated with an iron disorder or a genetic susceptibility to developing said disorder in a mammal

comprising the nucleic acid molecule of claims 49, 50, or 51.

53. A kit for the detection of the presence of a mutation in exon 2 of an HFE nucleic acid comprising a first oligonucleotide primer which is 5' to exon 2 and a second oligonucleotide primer is 3' to exon 2.